ClinGen Malignant Hyperthermia Susceptibility Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for RYR1 Version 2
This version specified for the following genes: *RYR1*
Expert Panel Page: https://www.clinicalgenome.org/affiliation/50038

Gene	Disease (MONDO ID)	Transcript
RYR1	Malignant Hyperthermia MONDO:0018493	NM_000540.3

Release Notes/Changes from v1:

(1) Revised PS4 such that at all strength levels an individual with two VUS/LP/P variants in *RYR1* cannot be considered as supporting pathogenicity of either variant.
(2) PS1 can be used at level moderate for previously classified likely pathogenic variant at the same codon with the same amino acid change.
(3) PM5 can be used at level supporting for previously classified likely pathogenic variant at the same codon, different amino acid change.
(4) PM1 should be downgraded to supporting when either PS1 or PM5 are used.

Modified ACMG criteria suggested for autosomal dominantly inherited RYR1/MH (see below for full explanations).

Criteria	Criteria Description	Specification
Pathogenic Criteria		
VERY STRONG CRITERIA		
PS2/PM6_ Very Strong	Each proven *de novo* case, 2 points, each assumed *de novo* case, 1 point, ≥8 points	Strength[a]
STRONG		
PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change • Previously established pathogenic variant must reach a classification of pathogenic without PS1	None
PS2/PM6_ Strong	Each proven *de novo* case, 2 points, each assumed *de novo* case, 1 point, a total of 4-7 points	Strength[a]
PS3	Well-established functional studies supportive of a damaging effect on protein function • Knock-in mouse showing MH reaction in response to RYR1 agonist AND increased sensitivity to RYR1 agonists in *ex vivo* tissue/cells	Strength[a], Disease-Specific

Related publication(s): PMID 33767344 **Date Approved:** March 1, 2022

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PS4	The prevalence of the variant in affected individuals significantly increased compared with the prevalence in controls • ≥7 MH case points. Probands with a personal or family history of an MH event are awarded 0.5 points, probands with a personal or family history[b] of a positive (MHS) IVCT/CHCT are awarded an additional 0.5 points. Probands with multiple variants in *RYR1* classified as VUS, likely pathogenic or pathogenic are not considered. Popmax in gnomAD ≤0.00006 • For variants with popmax MAF gnomAD >0.00006, an odds ratio of ≥18.7 when comparing MH case points to allele count in gnomAD can qualify. Popmax in gnomAD must be <0.0038	Strength[a], Disease-Specific
PP1_Strong	• Co-segregation with disease in ≥7 reported meioses	Strength[a]
MODERATE		
PM1	Located in a mutational hot spot and/or critical and well established functional domain • Residues 1-552 (N-terminal region) and 2,101-2,458 (central region) • PM1 should not be applied at a moderate weight with PS1/PM5, see PM1_Supporting	Disease-Specific
PM5	Missense change at an amino acid residue where a different missense variant previously determined to be pathogenic • Previously established pathogenic variant must reach a classification of pathogenicity without PM5 • Grantham score for alternate pathogenic variant must be less than for variant being assessed	None
PS1_ Moderate	Same amino acid change as a previously established likely pathogenic variant regardless of nucleotide change Previously established likely pathogenic variant must reach a classification of likely pathogenic without PS1	Strength[a]
PS2/PM6_ Moderate	Each proven *de novo* case, 2 points, each assumed *de novo* case, 1 point, a total of 2-3 points	Strength[a]
PS3_Moderate	Well-established functional studies supportive of a damaging effect on protein function • Increased sensitivity to RYR1 agonist in HEK293 *in vitro* assay, Ca^{2+} release significantly increased compared to WT, controls to include known pathogenic and benign variants, n≥3. • Three or more independent *ex vivo* studies all showing release of Ca^{2+} in response to RYR1 agonist • Knock-in mouse showing MH reaction in response to RYR1 agonist OR increased sensitivity to RYR1 agonists in *ex vivo* tissue/cells (but not both, which would be PS3_strong)	Strength[a], Disease-Specific

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PS4_Moderate	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls • 2-6 MH case points. Probands with a personal or family history[b] of an MH event are awarded 0.5 points, probands with a personal or family history of a positive (MHS) IVCT/CHCT are awarded an additional 0.5 points. Probands with multiple variants in *RYR1* classified as VUS, likely pathogenic or pathogenic are not considered. Popmax in gnomAD ≤0.00006 • For variants with popmax MAF in gnomAD >0.00006, an odds ratio of ≥4.33 when comparing MH case points to allele count in gnomAD can qualify. Popmax in gnomAD must be <0.0038	Strength[a], Disease-Specific
PP1_ Moderate	• Co-segregation with disease in 5-6 reported meioses	Strength[a]
PP3_ Moderate	Multiple lines of computational evidence support a deleterious effect on the gene or gene product • Use REVEL score of >0.85	Strength[a]
SUPPORTING		
PP1	Co-segregation with disease in 3-4 reported meioses	Strength[a]
PS2/PM6_ Supporting	Each proven *de novo* case, 2 points, each assumed *de novo* case, 1 point, a total of 1 point	Strength[a]
PS3_ Supporting	Well-established functional studies supportive of a damaging effect on protein function • Two independent *ex vivo* studies all showing release of Ca^{2+} in response to RYR1 agonist	Strength[a], Disease-Specific
PS4_ Supporting	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls • 1 MH case point. Probands with a personal or family history[b] of an MH event are awarded 0.5 points, probands with a personal or family history of a positive (MHS) IVCT/CHCT are awarded an additional 0.5 points. Probands with multiple variants in *RYR1* classified as VUS, likely pathogenic or pathogenic are not considered. Popmax in gnomAD ≤0.00006 For variants with popmax MAF in gnomAD >0.00006, an odds ratio of ≥2.08 when comparing MH case points to allele count in gnomAD can qualify. Popmax in gnomAD must be <0.0038	Strength[a], Disease-Specific
PM1_ Supporting	Located in a mutational hot spot and/or critical and well established functional domain • Residues 1-552 (N-terminal region) and 2,101-2,458 (central region), if PS1/PM5 applicable then PM1 should be used at supporting • Residues 4,631-4,991 (C-terminal region)	Strength, Disease-Specific

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PM5_ Supporting	Missense change at an amino acid residue where a different missense variant previously determined to be pathogenic • Previously established likely pathogenic variant can be considered supporting evidence, must reach a classification of likely pathogenic without PM5 • Grantham score for alternate likely pathogenic variant must be less than for variant being assessed	Strength, Disease-Specific
Benign Criteria		
STAND ALONE		
BA1	Popmax allele frequency >0.0038 (0.38%)	Disease-Specific
STRONG		
BS1	Popmax allele frequency >0.0008 (0.08%)	Disease-Specific
BS2	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age. • Two or more unrelated variant positive individuals with a negative IVCT/CHCT test	Disease-Specific
MODERATE		
BS2_Moderate	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age. • One variant positive individual with a negative IVCT/CHCT test	Strength[a], Disease-Specific
BS3_Moderate	Well-established functional studies show no damaging effect on protein function • Three or more independent *ex vivo* studies, **NO** significant release of Ca^{2+} in response to agonist	Strength[a], Disease-Specific
SUPPORTING		
BP2	Observed in *cis* with a pathogenic variant in any inheritance pattern	None
BP4	Computational evidence suggest no impact on gene or gene product, REVEL score of <0.5	Disease-Specific

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BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved	None
BS3_Supporting	Well-established functional studies show no damaging effect on protein function • No significant increased sensitivity to RYR1 agonist in an approved *in vitro* assay, Ca^{2+} release measured, n≥3 • One or two independent *ex vivo* studies, NO significant release of Ca^{2+} in response to agonist • Knock-in mouse showing no MH reaction in response to RYR1 agonist AND no increased sensitivity to RYR1 agonists in *ex vivo* tissue/cells	Strength[a], Disease-Specific

Key: Disease-Specific, Disease-specific modifications based on what is known about MHS; Strength, Increasing or decreasing strength of criteria based on the amount of evidence; N/A: not applicable for MHS; None, no changes made to existing criteria definitions; IVCT, *in vitro* contracture test; CHCT, caffeine-halothane contracture test.

[a]For criteria that can be assigned different levels of strength based on evidence, only the highest applicable strength level should be used. For example, if PS4 is met, then PS4_Moderate and PS4_Supporting are not used.

[b]Positive family history defined by variant positive family member with MH reaction and/or positive IVCT/CHCT.

[c]Sequence Variant Interpretation committee, ClinGen.

[d]Cardiomyopathy Expert Panel.[14]

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Explanation of modified ACMG criteria for autosomal dominantly inherited *RYR1*/MH.

VERY STRONG EVIDENCE OF PATHOGENICITY

PVS1	Null variant (nonsense, frameshift, canonical +/- 1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease. **MHS-*RYR1*:** PVS1 is not applicable. MHS is due to gain of function variants in *RYR1*.
PS2/PM6_ Very Strong	*De novo* in a patient with the disease and no family history. Counts BOTH proven and unproven *de novo* cases. Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity. **MHS-*RYR1*:** PS2/PM6 follow SVI recommendation for *de novo* criteria. Each proven de *novo* case gets 2 points, each unproven *de novo* case gets 1 point, PS2/PM6_Very Strong applied if ≥8 points. Note: The family history should be negative for MH events, central core disease, or exertional heat related illness.

STRONG EVIDENCE OF PATHOGENICITY

PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change. **MHS-*RYR1*:** PS1 is applicable as described. As with PM5, the initial variant determined to be pathogenic must reach an assessment of pathogenic without using this criterion (no double counting).
PS2/PM6_ **Strong**	*De novo* in a patient with the disease and no family history. Counts BOTH proven and unproven *de novo* cases. Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity. **MHS-*RYR1*:** PS2/PM6 follow SVI recommendation for *de novo* criteria. Each proven *de novo* case gets 2 points, each unproven *de novo* case gets 1 point, PS2/PM6_Strong applied if 4-7 points. Note: The family history should be negative for MH events, central core disease, or exertional heat related illness.

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PS3	Well-established *in vitro* or *ex vivo* functional studies or knock-in mouse studies supportive of a damaging effect on the gene or gene product. **MHS-*RYR1*:** • *In vitro* assays showing increased sensitivity to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage) can be used for PS3. All assays require appropriate controls such that likelihood ratios are ≥18.7. Historical data, when available, can be used to validate the assay. • MH reaction in response to RYR1 agonist in a knock-in mouse model, requires **BOTH** MH reaction in heterozygous animals AND increased sensitivity to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage) in an approved ex *vivo* assay using knock-in mouse tissues, Ca^{2+} release measured by fluorescence. Appropriate controls should be included.
PS4	The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls. **MHS-*RYR1*:**True case control studies do not exist in the *RYR1* literature with controls known to be negative for MHS. A modified PS4 is used for *RYR1* using MH case reports and data from gnomAD (Richards et al. Note 2). • PS4_Strong requires ≥7 MH case points, one point is awarded for a proband with a personal or family history (in a variant positive individual) of an MH event AND a positive IVCT or CHCT diagnostic test (MHS), 0.5 points are awarded for a proband with a reported MH event but without an IVCT or CHCT diagnostic test. Popmax MAF in gnomAD ≤0.00006. • For variants with popmax MAF in gnomAD >0.00006, and below BA1 cutoff of 0.0038, MedCalcs online calculator can be used to calculate the OR using case points from the literature, an approximation of 3,000 cases (6,000 alleles) reported in the literature and allele counts from gnomAD (MedCalc; https://www.medcalc.net/statisticaltests/odds_ratio.php). An OR of ≥18.7 is required for PS4_Strong. • Probands with multiple variants in *RYR1* classified as VUS, likely pathogenic or pathogenic are not considered.
PP1_Strong	Co-segregation with disease in multiple affected family members. **MHS-*RYR1*:** ≥7 meioses, only consider phenotype positive/variant positive individuals. To use PP1, no phenotype positive/variant negative individuals can be identified in a pedigree.

MODERATE EVIDENCE OF PATHOGENICITY

PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation. **MSH-*RYR1*:** Residue regions: 1-552 (N-terminal region) and 2,101-2,458 (central region) are thought to be critical functional domains for MHS. Should not be applied with PS1/PM5, see PM1_Supporting.

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PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC. **MHS-*RYR1*:** PM2 is not used alone.
PM3	For recessive disorders, detected in trans with a pathogenic variant Note: This requires testing of parents (or offspring) to determine phase. **MHS-*RYR1*:** PM3 is not applicable. MHS is inherited as an autosomal dominant trait with reduced penetrance.
PM4	Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants. **MHS-*RYR1*:** PM4 is not applicable. The majority of *RYR1* variants that are causative for MHS are missense variants.
PM5	Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before. **MHS-*RYR1*:** PM5 is applicable as described. As with PS1, the initial variant determined to be pathogenic must reach an assessment of pathogenic without using this criterion (no double counting). As well, the Grantham score difference for the initial variant determined to be pathogenic must be less than the Grantham score difference for the variant currently being assessed.
PS1_Moderate	Same amino acid change as a previously established likely pathogenic variant regardless of nucleotide change. **MHS-*RYR1*:** PS1_Moderate is applicable as described for PS1 with the exception that likely pathogenic variants can be used as evidence at a reduced weight. As with PM5, the initial variant determined to be likely pathogenic must reach an assessment of likely pathogenic without using this criterion (no double counting).
PS2/PM6_ **Moderate**	*De novo* in a patient with the disease and no family history. Counts BOTH proven and unproven *de novo* cases. Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity. **MHS-*RYR1*:** PS2/PM6 follow SVI recommendation for *de novo* criteria. Each proven *de novo* case gets 2 points, each unproven *de novo* case gets 1 point, PS2/PM6_Moderate applied for 2-3 points.

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	Note: Family history needs to be negative for MH events, central core disease, or exertional heat related illness.
PS3_Moderate	Well-established *in vitro* or *ex vivo* functional studies or knock-in mouse studies supportive of a damaging effect on the gene or gene product. **MHS-*RYR1*:** • *In vitro* assays showing increased sensitivity to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage) can be used for PS3_Moderate. All assays require appropriate controls such that likelihood ratios are ≥4.3. Historical data, when available, can be used to validate the assay. • Historical assay data for transfection studies in HEK293 cells supports using this assay at the moderate strength level. Result showing increased sensitivity to RYR1 agonist (halothane, caffeine, 4-CmC) supports pathogenicity. Result must show significant increase in Ca^{2+} release for agonist concentration (EC_{50}). Controls must include wildtype *RYR1* and known pathogenic variants that reach an assessment of LP/P without consideration of PS3. Assays should be run in triplicate. • Three or more independent *ex vivo* studies (tissues from unrelated individuals) all showing increased release of Ca^{2+} in response to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage). Ca^{2+} release measured by fluorescence. Appropriate controls included. Result must show significant increase in Ca^{2+} release at decreased agonist concentration. ○ Patient tissues considered useful for PS3 (and BS3) include patient myotubes, microsomal SR preps, and lymphoblasts. • MH reaction in response to RYR1 agonist in a knock-in mouse model, requires MH reaction in heterozygous animals OR increased sensitivity to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage) in an approved *ex vivo* assay using knock-in mouse tissues, Ca^{2+} release measured by fluorescence. Appropriate controls should be included.
PS4 _ **Moderate**	The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls. **MHS-*RYR1*:**True case control studies do not exist in the *RYR1* literature with controls known to be negative for MHS. A modified PS4 is used for *RYR1* using MH case reports and data from gnomAD (Richards et al. Note 2). • PS4_Moderate requires 2-6 MH case points, one point is awarded for a proband with a personal or family history (in a variant positive individual) of an MH event AND a positive IVCT or CHCT diagnostic test (MHS), 0.5 points are awarded for a proband with a reported MH event but without an IVCT or CHCT diagnostic test. Popmax MAF in gnomAD ≤0.00006. • For variants with popmax MAF in gnomAD >0.00006, and below BA1 cutoff of 0.0038, MedCalcs online calculator can be used to calculate the OR using case points from the literature, an approximation of 3,000 cases (6,000 alleles) reported in the literature and allele counts from gnomAD (MedCalc; https://www.medcalc.net/statisticaltests/odds_ratio.php). An OR of ≥4.33 is required for PS4_Moderate.

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	• Probands with multiple variants in *RYR1* classified as VUS, likely pathogenic or pathogenic are not considered.
PP1_Moderate	Co-segregation with disease in multiple affected family members. **MHS-*RYR1*:** 5-6 meioses, only consider phenotype positive/variant positive individuals. In order to use PP1 no phenotype positive/variant negative individuals can be identified in a pedigree.
PP3_Moderate	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.). **MHS-*RYR1*:** REVEL score of > 0.85 is considered evidence in support of pathogenicity. (PMID:27666373)

SUPPORTING EVIDENCE OF PATHOGENICITY

PP1	Co-segregation with disease in multiple affected family members. **MHS-*RYR1*:** 3-4 meioses, only consider phenotype positive/variant positive individuals. In order to use PP1 no phenotype positive/variant negative individuals can be identified in a pedigree.
PP2	Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease. **MHS-*RYR1*:** PP2 is not applicable. *RYR1* does not appear to be constrained for missense variation with a z-score of 1.92 in gnomAD.
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.). **MHS-*RYR1*:** Upgraded to PP3_Moderate.
PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology. **MHS-*RYR1*:** PP4 is not applicable, variants in *CACNA1S* also result in MHS.
PP5	Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation. **MHS-*RYR1*:** PP5 has been dropped from the ACMG framework for variant assessment.
PS2/PM6_	*De novo* in a patient with the disease and no family history. Counts BOTH proven and unproven *de novo* cases.

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Supporting	Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity. **MHS-*RYR1*:** PS2/PM6 follow SVI recommendation for *de novo* criteria. Each proven *de novo* case gets 2 points, each unproven *de novo* case gets 1 point, PS2/PM6_Supporting applied for 1 point. Note: The family history should be negative for MH events, central core disease, or exertional heat related illness.
PS3_Supporting	Well-established *in vitro* or *ex vivo* functional studies or knock-in mouse studies supportive of a damaging effect on the gene or gene product. **MHS-*RYR1*:** • *In vitro* assays showing increased sensitivity to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage) can be used for PS3_Supporting. All assays require appropriate controls such that likelihood ratios are ≥2.08. Historical data, when available, can be used to validate the assay. • Two independent *ex vivo* studies (tissues from unrelated individuals) all showing increased release of Ca^{2+} in response to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage). Ca^{2+} release measured by fluorescence. Appropriate controls included. Result must show significant increase in Ca^{2+} release at decreased agonist concentration. ○ Patient tissues considered useful for PS3 (and BS3) include patient myotubes, microsomal SR preps and lymphoblasts.
PS4_Supporting	The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls. **MHS-*RYR1*:**True case control studies do not exist in the *RYR1* literature with controls known to be negative for MHS. A modified PS4 is used for *RYR1* using MH case reports and data from gnomAD (Richards et al. Note 2). • PS4_Supporting requires one MH case point, one point is awarded for a proband with a personal or family history (in a variant positive individual) of an MH event AND a positive IVCT or CHCT diagnostic test (MHS), 0.5 points are awarded for a proband with a reported MH event but without an IVCT or CHCT diagnostic test.). Popmax MAF in gnomAD ≤0.00006. • For variants with popmax MAF in gnomAD >0.00006, and below BA1 cutoff of 0.0038, MedCalcs online calculator can be used to calculate the OR using case points from the literature, an approximation of 3,000 cases (6,000 alleles) reported in the literature and allele counts from gnomAD (MedCalc; https://www.medcalc.net/statisticaltests/odds_ratio.php). An OR of ≥2.08 is required for PS4_Supporting. • Probands with multiple variants in RYR1 classified as VUS, likely pathogenic or pathogenic are not considered.

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PM1_Supporting	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation. **MSH-*RYR1*:** Residue region: 4,631-4,991 (C-terminal region) is thought to be a critical functional domain for MHS. Variants in this domain have been identified in MH and CCD. • Residues 1-552 (N-terminal region) and 2,101-2,458 (central region), if PS1/PM5 used PM1 should be used at supporting
PM5_Supporting	Missense change at an amino acid residue where a different missense change determined to be likely pathogenic has been seen before. **MHS-*RYR1*:** PM5_Supporting is applicable as described for PM5 with the exception that likely pathogenic variants can be used as evidence at a reduced weight. As with PS1, the initial variant determined to be likely pathogenic must reach an assessment of likely pathogenic without using this criterion (no double counting). As well, the Grantham score difference for the initial variant determined to be likely pathogenic must be less than the Grantham score difference for the variant currently being assessed.

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STAND ALONE EVIDENCE OF BENIGN IMPACT

BA1	Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes, or ExAC. **MHS_*RYR1*:** An allele frequency of ≥ 0.0038 (0.38%) is used as a cut off based on popmax MAF in gnomAD (outbred population). Calculating a stand-alone filtering frequency for MHS-RYR1 is complicated as neither the frequency nor the penetrance of MHS is well understood. Based on reduced penetrance and the requirement of a triggering event the incidence of MH events is expected to be lower than the incidence of MHS. Studies have reported an incidence of MH events as low as 1 in 10,000 to 1 in 250,000 anesthesias (PMID: 26709912). Variants in *RYR1* are reported to account for ~ 76% of cases (PMID: 30236257). Penetrance for MH is not well understood, we instead substituted a value of 1%, as it is a reasonable boundary between the penetrance of a mendelian disorder variant and that of a risk allele. Maximum Prevalence of MHS = Prevalence of MH events / Penetrance (1 event/10,000 children) / (1 event/100 causative alleles) 1 causative allele/100 children MHS prevalence (0.01) * *RYR1* contribution (0.76) * Allele conversion (0.5) = 0.0038

STRONG EVIDENCE OF BENIGN IMPACT

BS1	Allele frequency is greater than expected for disorder. **MHS-*RYR1*:** An MH all allele frequency of ≥ 0.0008 (0.08%) is used as a cut off for popmax MAF in gnomAD (outbred population). Disease prevalence as explained for BA1. Disease prevalence (0.01) * Maximum single *RYR1* variant contribution (0.16) * Allele conversion (0.5) = 0.0008
BS2	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age. **MHS-*RYR1*:** The absence of an MH reaction in a healthy individual cannot be used for BS2 due to reduced penetrance. BS2 is applicable if two or more unrelated variant positive individuals have negative results for either the IVCT or CHCT.

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BS3	Well-established *in vitro* or *ex vivo* functional studies or knock-in mouse studies show no damaging effect on protein function. **MSH-RYR1:** BS3 downgraded to BS3_Supporting for all negative data.
BS4	Lack of segregation in affected members of a family **MSH-*RYR1*:** BS4 is not applicable. Phenotype for MHS is routinely determined based on the vitro contraction test (IVCT) that has a false positive rate of approximately 6% (PP1) or the caffeine-halothane contracture test (CHCT). As the phenotype in individuals who have not experienced an MH crisis cannot be reliably determined BS4 is not utilized.

MODERATE EVIDENCE FOR BENIGN IMPACT

BS2_Moderate	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age. **MHS-*RYR1*:** The absence of an MH reaction in a healthy individual cannot be used for BS2 due to reduced penetrance. BS2_Mod is applicable if a single variant positive individual has a negative result for either the IVCT or CHCT diagnostic tests.
BS3_Moderate	Well- established *in vitro* or *ex vivo* functional studies or knock-in mouse studies show no damaging effect on protein function. **MHS-*RYR1*:** • Three or more independent *ex vivo* studies all showing **NO** significant increase in release of Ca^{2+} in response to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage). Ca^{2+} release measured by fluorescence. Appropriate controls included. Result must show lack of significant increase in Ca^{2+} release at decreased agonist concentration.

SUPPORTING EVIDENCE FOR BENIGN IMPACT

BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease. **MHS-*RYR1*:** BP1 is not applicable. MH is caused primarily by missense variants in *RYR1*.
BP2	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in cis with a pathogenic variant in any inheritance pattern. **MHS-*RYR1*:** BP2 is applicable for variants shown to be in cis with a known pathogenic variant.

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BP3	In-frame deletions/insertions in a repetitive region without a known function **MHS-*RYR1*:** BP3 is not applicable. *RYR1* does not have repetitive regions without known function.
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.). **BP4 cannot be used in isolation, at least one other criteria must apply to a variant in order to utilize BP4. If used in isolation it can define a variant as likely benign, it was determined by the VCEP that a variant should not be assessed to be likely benign based solely on computational data.** **MHS-*RYR1*:** REVEL score < 0.5 is considered evidence against pathogenicity.
BP5	Variant found in a case with an alternate molecular basis for disease. **MHS-*RYR1*:** BP5 is not applicable as individuals have been described with MHS and two pathogenic variants in RYR1.
BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation. **MHS-*RYR1*:** BP6 has been dropped from the ACMG framework for variant assessment.
BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved. **MHS-*RYR1*:** BP7 applicable as described.
BS3_Supporting	Well- established *in vitro* or *ex vivo* functional studies or knock-in mouse studies show no damaging effect on protein function. **MHS-*RYR1*:** • **NO** significant increase in Ca^{2+} release in response to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage) in an *in vitro* transfection assay (HEK293, CHO, dyspedic myotubes), Ca^{2+} release measured by fluorescence. Both positive and negative controls included to include variants previously identified as pathogenic and benign. Result must show lack of a significant increase in Ca^{2+} release. Assay must be run in triplicate. • One or two independent *ex vivo* studies all showing **NO** significant increase in release of Ca^{2+} in response to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage). Ca^{2+} release measured by fluorescence. Appropriate controls included. Result must show lack of significant increase in Ca^{2+} release at decreased agonist concentration.

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	○ *Ex vivo* studies using patient derived samples need to be interpreted with the understanding that unidentified variants may be present. Patient tissues considered useful for BS3 include patient myotubes, microsomal SR preps and lymphoblasts. • **NO** MH reaction in response to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage) in a knock-in mouse model AND **NO** significant increase in sensitivity to RYR1 agonist (halothane, caffeine, 4-CmC, KCl, voltage) in knock-in mouse tissues, Ca^{2+} release measured by fluorescence. Appropriate controls included.

Key: IVCT, in vitro contracture test; CHCT, caffeine halothane contracture test.

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ClinGen Malignant Hyperthermia Susceptibility Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for RYR1 Version 2

This version specified for the following genes: *RYR1*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50038

RULES FOR COMBINING PATHOGENIC CRITERIA

Bayesian Classification Framework as suggested by Tavtigian et al. 2018 is utilized.

Sum all criteria that are applicable to the variant. Calculate Odds of Pathogenicity using formula below, calculate posterior probability, use posterior probability to determine pathogenicity.

Odds of Pathogenicity = $2.1^{\#\text{Total Supporting}} * 4.3^{\#\text{Total Moderate}} * 18.7^{\#\text{Total Strong}} * 350^{\#\text{Total V Strong}} * 0.4808^{\#\text{Benign Supporting}} * 0.2326^{\#\text{Benign Moderate}} * 0.0535^{\#\text{Benign Strong}}$

Posterior Probability = (Odds Path * 0.1) / (Odds Path - 1) * 0.1+1)

Assignment of Pathogenicity based on Posterior Probability:

Posterior Probability < 0.001	Benign
Posterior Probability ≥ 0.001 < 0.1	Likely Benign
Posterior Probability ≥ 0.10 < 0.9	VUS
Posterior Probability ≥ 0.9 to < 0.99	Likely Pathogenic
Posterior Probability ≥ 0.99	Pathogenic

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